Physiological Responses Of Cotton Plant (*Gossypium Hirsutum* L.) To Foliar Sprayed Folic And Salicylic Acid Under Drought Conditions

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Abstract
The current study was conducted from February 2023 to August 2023 at a local farm in the Biology Department, College of Science, University of Zakho in the Kurdistan Region of Iraq. It aimed to examine the effects of drought and the application of salicylic and folic acid on some physiological parameters of two cotton (*Gossypium hirsutum* L.) cultivars (MAY 505 and MAY 455). For this reason, a randomized complete block design (RCBD) was used to assess the physiological aspects of the cotton plant. Both cotton cultivars were treated with salicylic acid (SA) at concentrations of 0, 1, and 2 mM, and folic acid (FA) at 0, 5, and 10 mM, separately and in combination, and subjected to three drought periods as (continuous irrigation (Ci), 10, and 15 days thirst). The results indicated that proline content was the highest in plants treated with (1 mM SA) at (33.75 µg/mg) in MAY 505 and (28.29 µg/mg) in MAY 455 under similar drought conditions. Further, plants sprayed with (5 mM FA) showed the highest chlorophyll content at (46.82 SPAD value) for MAY 505 and (50.77 SPAD value) for MAY 455. The application of (2 mM SA) scored the highest increase in P, K, and Mn, while the applied (10 mM FA) showed the highest increase in N and Cu. Cultivar MAY 505 was shown to be more responsive in addressing drought periods than MAY 455.

Keywords: *Gossypium hirsutum*, Drought, Salicylic acid, Folic acid, proline.

Introduction

Cotton plant is cultivated globally as an industrial crop, primarily for the clothing and weaving industries where it serves as a key component. Additionally, cotton produces significant quantities of animal feed and oil, leading to a consistently rising demand (Gul *et al*., 2022).

Water shortage can significantly impact plant growth and yield, especially in arid and semi-arid areas. Drought stress can lead to crop losses and affect physiological processes in plants (Ghaderi *et al*., 2015; Blum, 2017). It can alter physiological processes and osmolytes, such as proline content, chlorophyll content, soluble sugars, mineral uptake, and antioxidant activities (Nikolaeva *et al*., 2010; Dawood *et al*., 2014; Blum, 2017; Hasanuzzaman *et al*., 2012).

Crop production is greatly affected by environmental fluctuations, particularly drought due to climate change. Understanding how plants respond to water deficit conditions is crucial (Dalil and Golezani, 2012; Abbas *et al*., 2021). Different plants adjust to water scarcity differently, with some altering their leaf structures and accumulating osmolytes to survive (Hameed *et al*., 2012; Chen and Zhang, 2016; Nayak *et al.*, 2022).

Drought also affects nutrient uptake, leading to stunted growth and reduced yield (Kim *et al*., 2019; Gaafar *et al*., 2022). While advancements in genetic engineering and biotechnology aim to create drought-resistant plants, concerns about food security persist (Hayat *et al.* 2010; Feng et al., 2023; Itaf *et al*., 2023). Secure approaches such as the exogenous application of folic acid and salicylic acid as growth regulators and enhancers to resist abiotic stresses have shown promising results Ibrahim *et al*., (2020). This study aims to uncover the combined role of foliar spray of folic acid and salicylic acid in enhancing physiological measures in two cotton cultivars under drought stress.

Materials and methods

Study area

The study was conducted in the Zakho district at the College of Science, University of Zakho. The Zakho district is situated northwest of Duhok governorate at latitude 37.1505 N and longitude 42.6727 E, with an elevation of 440 meters above sea level as shown in Figure (1).

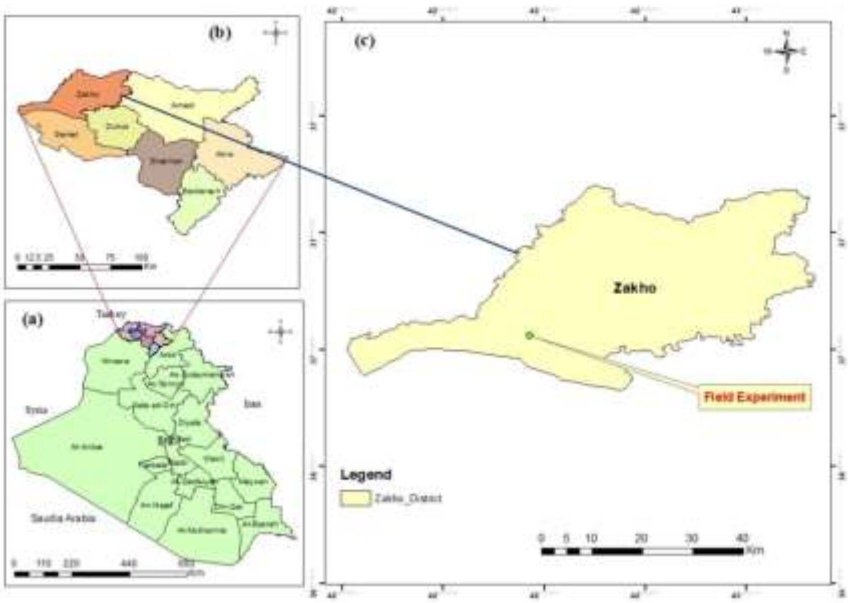

Figure 1. The location of the study.

Materials and methods

Field preparation and experimental units

The field was plowed in mid-February 2023 and allowed to dry in the sun for 15 days. Subsequently, the soil was harrowed and arranged into blocks based on the Randomized Complete Block Design (RCBD). Three concentrations of Folic acid (0 mM, 5 mM, 10 mM) and salicylic acid (0 mM, 1 mM, 2 mM) were prepared for spraying in addition to three water-stressed levels (continuous irrigation (Ci), 10 and 15 days of stopping irrigation) to investigate their effects on some physiological parameters on two cotton cultivars (May 505 and May 455).

Seed germination and treatment application

After preparing the field, cotton seeds were soaked in water overnight before being sown in holes with a depth of 1.5 inches. Foliar treatments were applied at night to minimize solution evaporation, targeting two growth stages: during seedling emergence with four leaves and at the onset of flowering.

Proline estimation

Free proline was estimated following the method of Bates *et al*. (1973). A 0.5 g sample of cotton leaf was homogenized in 10 ml of 3% aqueous sulphosalicylic acid and incubated for 3 hours. The homogenate was centrifuged at 15,000 rpm for 10 minutes, and the supernatant was filtered through Whatman filter paper (2.0). In a new tube, 1 ml each of acid ninhydrin and glacial acetic acid were added to the supernatant and mixed, then incubated in a water bath at 95°C for 1 hour. The tubes were then cooled on ice to halt the reaction. Finally, 1 ml of the mixture and 3 ml of toluene were added to a cuvette, and the absorbance was measured using a spectrophotometer at 530 nm. The following curve was used to calculate proline content.

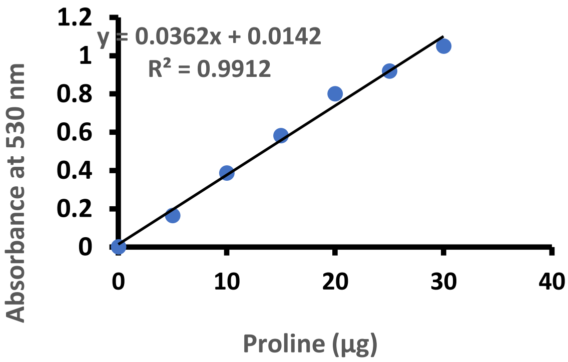

Figure 2. Proline standard curve.

Total chlorophyll content

Chlorophyll content was measured using a chlorophyll meter (SPAD meter 507, Japan). For each replication, three plants were selected. Five leaves from each plant were taken to measure the chlorophyll content. Four different spots were tested from each leaf using a chlorophyll meter then their average was obtained.

Total soluble sugar assessment

The available soluble sugar content was measured using a Brix refractometer. Fresh leaves were squeezed to extract their juice, which was placed on the Brix prism for 4 seconds to determine total soluble sugar (Braddock, 2011).

Mineral Composition

Macronutrients, such as nitrogen, phosphorus, and potassium, were determined using fresh cotton leaves that were harvested and sun-dried. The dried leaves were ground, and 0.5 g of the leaf powder was digested in concentrated sulfuric acid for 24 hours. Potassium was measured with a flame photometer (JENWAY PFP7, United Kingdom) as described by APHA (1998).

Following the protocol by Ryan et al. (2001), nitrogen content was assessed by extracting the leaf samples and applying the Kjeldahl method. Phosphorus was analyzed using the molybdenum blue method described by Williams and Williams (1965), while micronutrients such as cobalt, manganese, nickel, and copper were quantified using atomic absorption spectrophotometer (PerkinElmer, Inc., USA).

Oil content

To estimate the oil content of cotton seeds, the seeds were dried, roasted, peeled, and ground into a fine powder, which was then placed in a Soxhlet apparatus. It was operated for approximately 5 hours using N-hexane as a solvent to extract the oil. The extracted oil was then transferred to an evaporator to separate it from the solvent, following this, the oil was collected in a separate container and weighed in grams (Hutchins, 1953). The oil percentage was calculated using the following formula.

Oil percentage = weight of oil/weight of sample * 100

Total protein content

The total protein content in plant tissues was estimated by converting the total nitrogen amount using a Protein Conversion Factor of 6.25. This factor is derived from the assumption that the nitrogen content in proteins averages about 16%, leading to the estimation that protein content equals 100/16, or 6.25 times the nitrogen content. More specifically, a conversion factor for cottonseeds of (5.30) was suggested by (Jones, 1931). The calculation is represented by the formula:

Total Protein Content = Total Nitrogen Content × 5.3

Statistical analysis

The collected data was analyzed with SPSS (2019) to perform statistical analyses, including one-way, two-way, and three-way ANOVA, alongside descriptive statistics utilizing the CRD design. Parameter means were estimated using the Duncan test (Duncan, 1955).

Results

In this study, two cotton cultivars *G. hirsutum* (MAY 505 and MAY 455) were used to study some physiological changes under drought conditions. Significant variations were observed in physiological parameters across the treatments used and between the two selected cultivars.

1- Proline content (µg/mg)

Proline is a crucial amino acid accumulate by plants undergoing drought periods. In this study, proline accumulation significantly increases with increased drought period. For example, MAY 505 significantly produced more proline (16.1µg/mg) than MAY 455 at (13.81µg/mg). This indicates that the cultivar type responds differently to environmental change. Figure (3) illustrates the effect of drought periods on proline content revealing that plants undergoing 15 days of drought accumulated more proline (22.9 µg/mg) than 10 days of drought (12.58 µg/mg) and continuous irrigation regime (9.39 µg/mg).

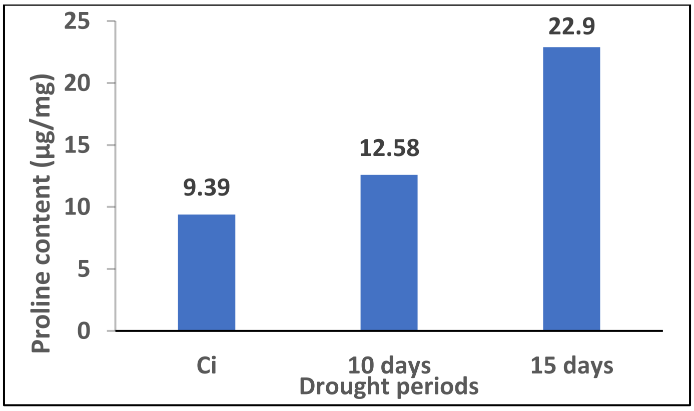

Figure 3. Shows the effect of drought on proline buildup under different drought periods.

Likewise, the SA spraying significantly affects proline content at the highest value of (23.371µg/mg) when plants were treated with 1mM SA followed by (18.74 µg/mg) in plants sprayed with (2mMSA+10mM FA) which was greater than untreated plants. Therefore, it can be noted that different treatment application affects the amount of proline production in plants (see Figure 4).

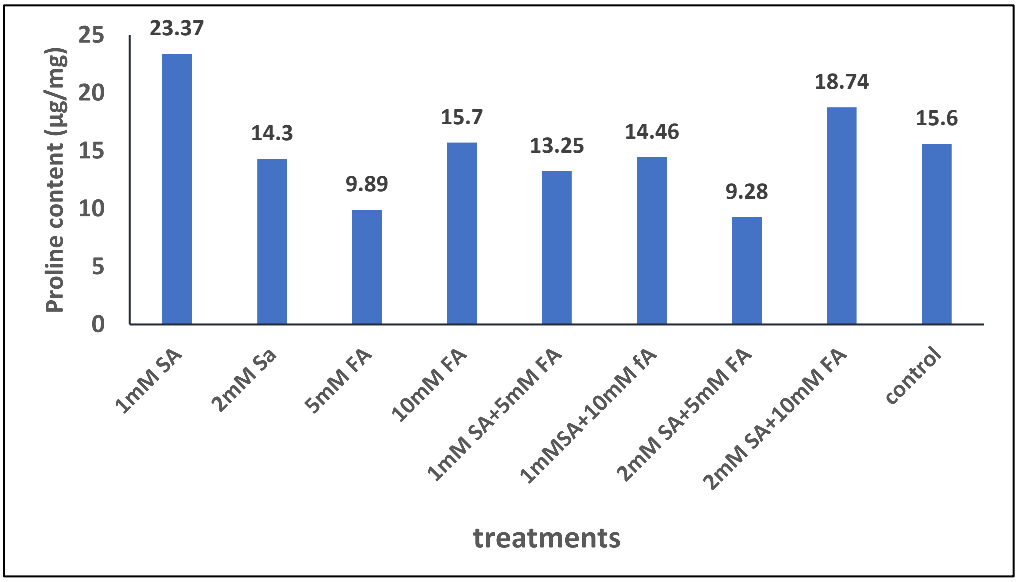

Figure 4. shows the effect of spraying treatments on proline accumulation.

The interactive effect of cultivar vs drought periods displays significant alterations among drought periods. For example, 15 days of drought showed the highest proline content at (24.26 µg/mg) in MAY 505 compared to MAY 455 at lower proline content at (21.54 1µg/mg) (see Figure 5).

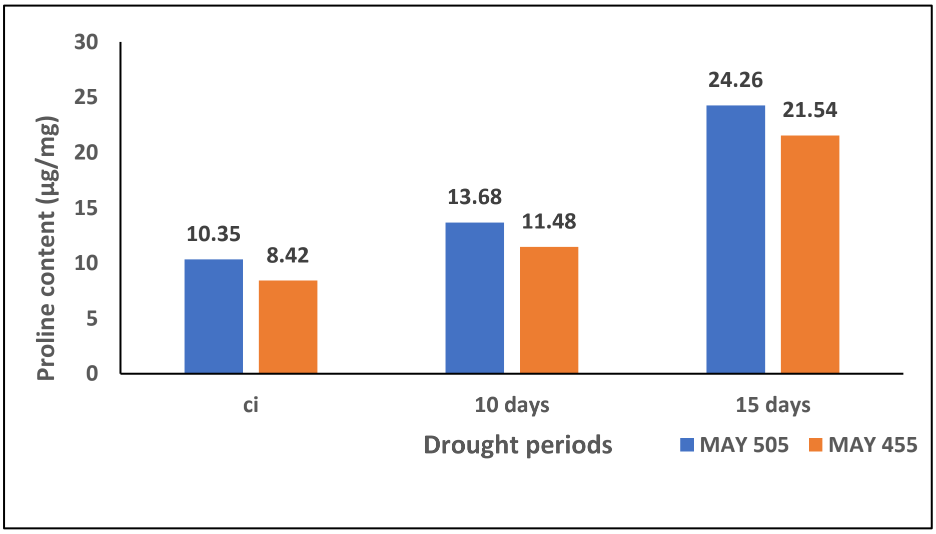

Figure 5. shows the effect of cultivar vs drought on proline content.

The effect of spraying treatment on cultivars shows a clear pattern of response in both studied cultivars. For instance, MAY 505 was found to be more effective than MAY 455 in storing proline content at (25.63 µg/mg) and (21.11 µg/mg) respectively. Thus, treatment application greatly enhanced MAY 505 in accommodating drought periods by producing greater proline than MAY 455. More to the point, the interactive effect between drought and treatments applied demonstrates that the more the plants experience drought duration, the more they accumulate proline content. In this instance, plants sprayed with 1mM SA shows the highest buildup of proline among all treatments applied. In this circumstance, crops that undergo drought stress would be better treated with 1mM SA to mitigate drought tolerance.

2- Total chlorophyll content (SPAD value)

SPAD (Soil Plant Analysis Development) meters are used to indirectly measure the amount of chlorophyll in plant leaves. These handheld devices measure the greenness of leaves, which is a good indicator of chlorophyll content. The level of drought has a significant impact on the SPAD values of the two cotton cultivars. The interaction between drought vs treatment revealed a slight variation in SPAD values. For example, plants treated with 2 mM SA+ 10 mM FA produced chlorophyll content at (41.4) under continuous irrigation whereas this value slightly decreased to (38.7) in plants exposed to 10 days of drought and then increased to (39.9) in plants experienced 15 days of water deficit under similar drought conditions (see Figure 6).

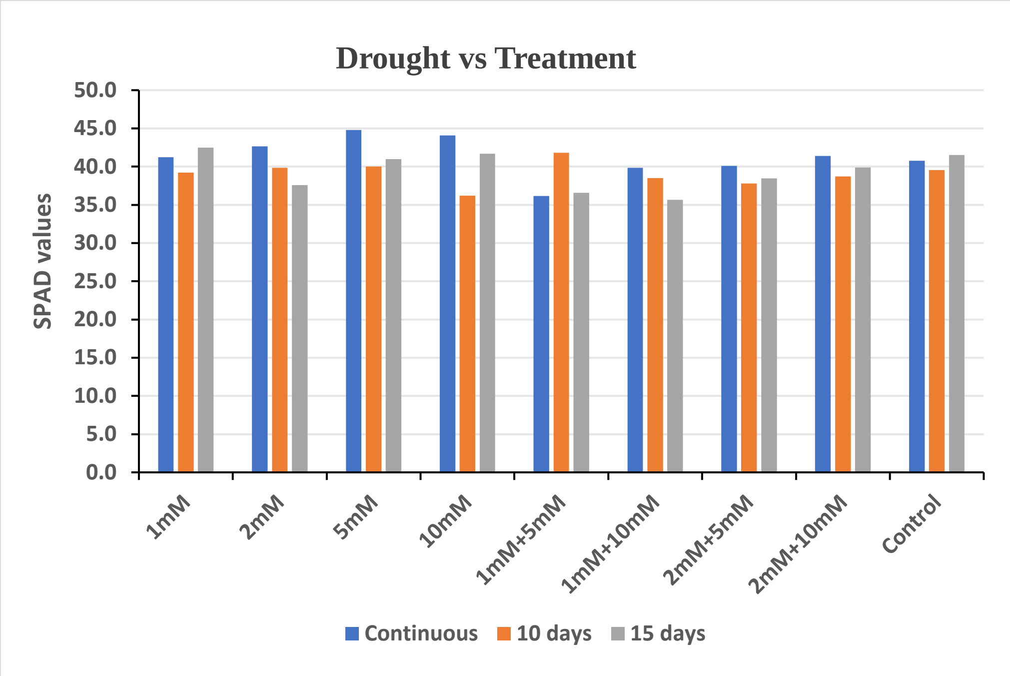

Figure 6. shows the interaction between drought vs treatment.

The amount of chlorophyll decreased as the plants experienced 10 days of water deprivation. Both cultivars showed a similar decrease in SPAD values during this period. However, cultivar MAY 505 was slightly more successful in producing more chlorophyll. Statistical analysis of the data indicated that the treatment had an insignificant effect on the chlorophyll content based on the SPAD readings. Overall, the drought harmed chlorophyll content, with both 10 and 15 days of drought leading to a decrease in chlorophyll (see Figure 7).

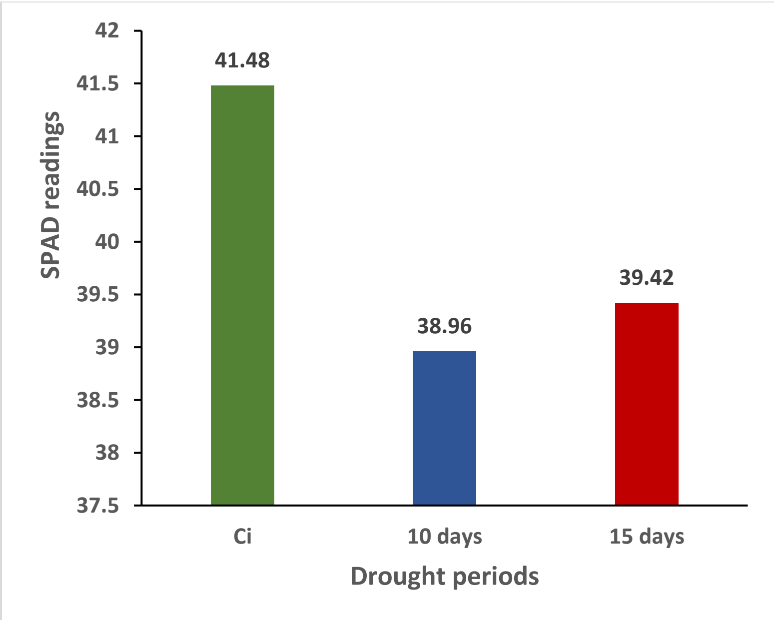

Figure 7. shows the effect of drought periods on chlorophyll content.

The interaction between cultivar and drought revealed a significant difference in SPAD readings, Specifically, cultivar MAY 505 produced less chlorophyll than its counterpart (Figure 8) and was more negatively impacted by drought compared to MAY 455. Thus, cultivar MAY 455 is less susceptible to drought stress regarding chlorophyll content.

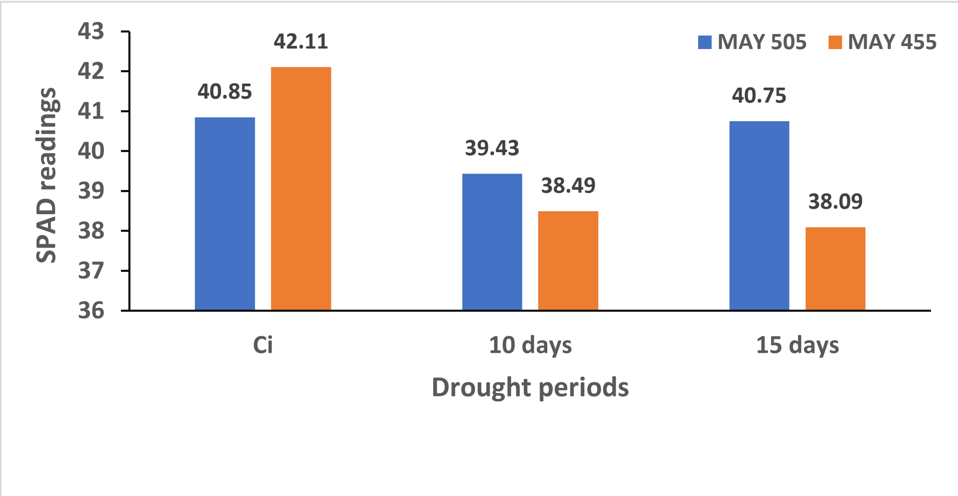

Figure 8. illustrates the SPAD values in the interaction effect between cultivar vs. drought period.

3. Oil content (%)

The exogenous application of SA and FA used in this study significantly affected the amount of oil produced. For instance, plants sprayed with a combined of (1 mM SA+ 5 mM FA) significantly increased the oil content of seeds by (10.89%) which is slightly less than the oil amount produced by control plants. On the other hand, the least effective treatment yielded (8.87%) of oil in plants treated with 5 mM FA. The effect of cultivar on oil content clearly shows significant results. MAY 505 recorded the highest amount of oil at (12.99%), while MAY 455 produced less oil at (6.62%). This indicates that cultivar MAY 505 is more tolerant to drought stress and more influential in producing oil under the same drought environments.

Oil content significantly varied in the three drought periods where continuously irrigated plants increased the amount of oil at (12.44%). However, this amount was reduced to (10.04%) in plants exposed to 10 days of water deficiency and further decreased to (6.94%) in plants experiencing 15 days of water deficiency. These results refer to the point that by increasing drought periods, the plant produces less oil content. These results were similarly observable in the interaction effect of drought vs cultivar. In this instance, in cultivar MAY 505, plants experiencing 10 days of dehydration produced (13.76%) of oil whereas cultivar MAY 455 made (6.31%) of oil under the same conditions (table 1). These results elucidate that cultivar MAY 505 is more fitted to drought periods as compared to cultivar MAY 455.

In the interactive effect of cultivar vs treatment, the obtained results indicate that the effect of FA and SA on oil content is influenced by the cultivar. For example, MAY 505 differently respond to similar treatment compared to MAY 455 scoring (15.98%) of oil while only (5.8%) of oil for MAY 455. Furthermore, the interactive effect between drought vs treatment demonstrates that oil content is not only affected by drought but also by specific treatment practiced. In this regard, the effect of treatment was more profound under 15 days of drought at (4.93%) when plants were sprayed with 5 mM FA than under 10 days of drought and continuous irrigation (table 1).

Table 1. summarizes the effect of FA and SA on oil content in cotton plants under three drought periods and their interactions.

Cultivar	Drought period	Treatment									Cultivar x drought	Cultivar effects
		Salicylic acid		Folic acid		Salicylic acid + Folic Acid				Control		
		1mM	2mM	5mM	10mM	1mM+ 5mM	1mM+ 10mM	2mM+ 5mM	2mM+ 10mM			
May 505	Ci	16.34 f	8.97 e	16.83 e	14.21 k	17.5 b	11.4 r	9.12 d	17.8 a	17.42 c	14.40 A	12.99 a
	10 days	15.82 h	17.08 d	12.55 n	13.63 l	14.34 j	9.57 b	15.71 i	12.68 m	12.47 p	13.76 b	
	15 days	9.97 w	11.08 s	7.37 i	9.81 z	16.09 g	10.94 t	9.69 a	9.87 y	12.5 n	10.81 c	
May 455	Ci	7.73 g	9.28 c	10.79 u	9.56 b	9.92 x	10.49 v	12.53 o n	12.56 n	11.47 q	10.48 d	6.62 b
	10 days	4.75 o	7.13 j	3.18 s	4.04 q	5.55 n	9.87 y	8.23 f	7.43 h	6.61 k	6.31 e	
	15 days	0.87 W	1.86 v	2.49 t	4.28 p	1.94 u	2.46 t	4.11 q	3.54 r	6 l	3.06 f	
Cultivar x treatment	May 505	14.04 b	12.38 e	12.25 f	12.55 d	15.98 a	10.64 h	11.51 g	13.45 c	14 b	Effect of drought	

	May 455	4.45 q	6.09 m	5.49 p	5.96 n	5.8 o n	7.61 l	8.29 i	7.84 k	8.03 j	
Drought x Treatment	**Ci**	12.04 f	9.125 r	13.81 c	11.89 h	13.71 d	10.95 i	10.85 j	15.18 a	14.45 b	12.44 a
	10 days	10.29 k	12.11 e	7.87 t	8.84 s	9.95 m	9.72 o	11.97	10.055	9.54	10.04 b
	15 days	5.42 y	6.47 x	4.93 z	7.05 u	9.02 s	6.7 w	6.9 v	6.71 w	9.25 q	6.94 c
Effect of treatment		9.25 f	9.23 f	8.87 h	9.26 e	10.89 b	9.12 g	9.89 d	10.65 c	11.08 a	

* Means with the same letters shows non-significant values based on Duncan's test at 0.05 level.

4- Total soluble sugar (°Brix)

The total soluble sugar (TSS) measures the amount of different sugars in a solution, like glucose, fructose, and sucrose. It's usually shown as a percentage or grams per liter. Brix, on the other hand, only measures the amount of sucrose. Hence, a 20 Brix solution has 20 grams of sucrose in every 100 grams of the liquid.

Drought stress causes the plants to accumulate total soluble sugar (TSS) as part of their physiological response to mitigate drought tolerance. In this study, investigation on TSS in cotton crops under drought stress showed that cultivars MAY 505 and MAY 455 responded similarly in accumulating the amount of TSS. For this reason, the cultivars show non-significant variation in response to treatments.

The effect of treatment displayed no significant alteration in all treatments applied except for the combined treatment of (2 Mm SA+10 mM FA) which recorded the highest amount of TSS at (21.13 °Brix). This indicates that this combination of treatments can be recommended to be applied on plants undergoing deficit irrigation periods to resist drought stress. In the meantime, the effect of drought exhibited no significant change in accumulating TSS in both controlled plants and plants experiencing 10 days of drought at (20.42 and 20.43 °Brix) respectively (figure 8 A). Conversely, plants exposed to 15 days of thirst, showed a significant change in accumulating the highest amount of TSS at (21.01 °Brix). This result indicates that the longer the plants are exposed to drought periods, the more TSS they accumulate to lessen the negative impact of drought on plant activities.

The interactive effect of drought on both cultivars showed similar effects in continuously irrigated plants and in plants facing 10 days of drought (see Figure 9a). However, this effect caused the plants to accumulate more TSS in response to prolonged drought after 15 days of water deprivation. MAY 505 stored the highest amount of TSS at (21.1 °Brix) as shown in Figure (9 b). In this regard, TSS along with other osmo-protectants such as proline, jointly collaborate in overcoming drought stress.

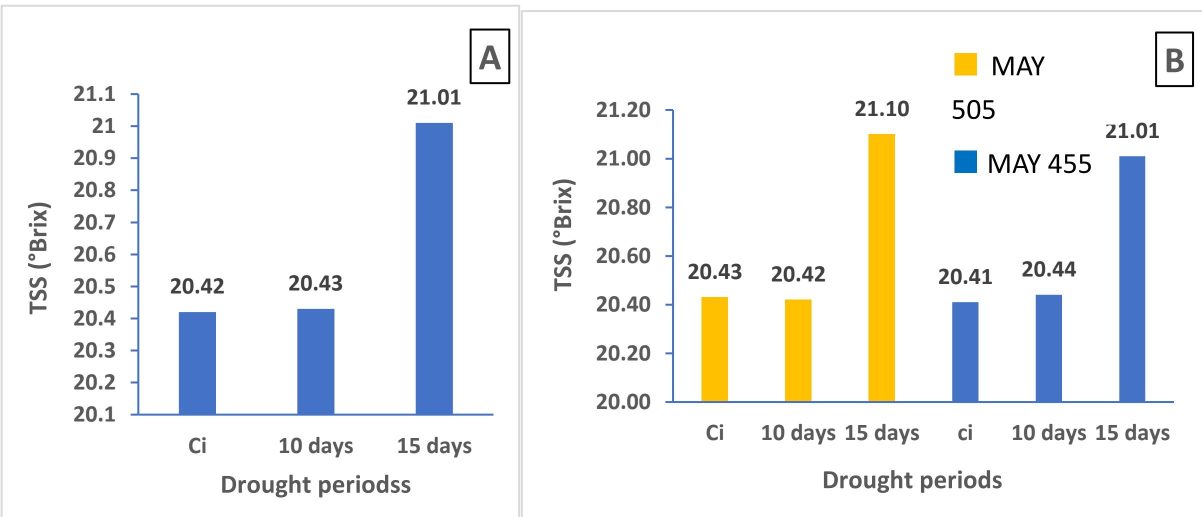

Figure 9. shows the effect of SA and FA on TSS in cotton plants under drought stress; (A) the effect of drought periods on total dissolved sugar accumulation. (B) the interaction between drought periods and cultivars.

About the interaction effect, the 15 days of drought significantly increased the amount of TSS at (21.01 °Brix) when compared to 10 days of drought and continuous irrigation regime. The relation between cultivars and treatments showed that the combined treatment (2 mM SA+ 10 mM FA) recorded the highest levels of TSS buildup at (21.13 °Brix) in both cultivars followed by (20.93 °Brix) in MAY 455 and (20.83 °Brix) in MAY 505.

The buildup of TSS is a crucial agent taking a vital part in drought tolerance. In the interaction between drought and treatment, a clear association was observed. For example, the highest increase in TSS levels was estimated in plants treated with (2 mM SA+ 10 mM FA) at (21.4 °Brix) in plants that experienced 15 days of drought which shows more TSS buildup as compared to 10 days of drought and control plants.

5- Mineral composition

Plants adapt to drought stress through mineral accumulation, which climate change can affect. This study examines the uptake and accumulation of nitrogen (N), phosphorus (P), potassium (K), manganese (Mn), cobalt (Co), copper (Cu), and nickel (Ni) during different drought periods. Statistical analysis reveals significant increases in K and Co for cultivar MAY 505, and in Cu and Ni for cultivar MAY 455, while Mn, N, and P show no significant changes in either cultivar. These findings indicate that the cultivar's impact on mineral uptake is ineffective.

The data presented in Table 2 shows the impact of different treatments on mineral composition. It indicates that plants sprayed with 2 mM SA exhibited the highest increase in P, K, and Mn compared to other treatments. On the other hand, plants treated with 10 mM FA showed the highest mineral acquisition in N and Cu. This finding is consistent with a study by Li et al. in 2023, which investigated the effects of drought stress on *Cunninghamia lanceolata* plants. Overall, it is clear that the application of 2 mM SA is the most effective among all the treatments. Additionally, the combined effect of SA and FA (1mM SA + 10mM FA) was found to be the most effective in increasing mineral concentrations in leaf tissues.

Table 2. illustrates the treatment effect on mineral accumulation in cotton plants. Similar letters represent non-significant values.

Minerals	Salicylic acid		Folic acid		Salicylic acid + Folic acid				Control
	1mM	**2mM**	**5mM**	**10mM**	**1mM + 5mM**	**1mM + 10mM**	**2mM + 5mM**	**2mM + 10mM**	
N (%)	2.28 cd	2.52 ab	2.20 cd	2.61 a	2.44 a-c	2.45 a-c	2.22 cd	2.59 a	2.15 d
P (mg/Kg)	682.71 b	838.6 a	460.23 d	464.71 d	572.77 c	803.84 a	687.49 b	449.79 d	726.87 b
K (%)	1.78 c	4.97 b	1.66 f	1.62 h	1.72 e	1.61 h	1.63 g	1.76 d	1.92 a
Mn (mg/L)	0.621 c	0.76 a	0.518 e	0.452 f	0.651 b	0.629 c	0.467 f	0.545 d	0.551 d
Co (mg/L)	0.54 b	0.49 c	0.42 d	0.46 c	0.48 c	0.55 b	0.41 d	0.59 a	0.55 b
Ni (mg/L)	0.13 b	0.24 a	0.25 a	0.051 e	0.06 de	0.12 cd	0.11 cd	0.02 e	0.18 b
Cu (mg/L)	0.053 a-c	0.042 bc	0.056 ab	0.073 a	0.037 bc	0.073 a	0.029 bc	0.052 a-c	0.025 c

The accumulation of Nitrogen and Phosphorus significantly increased in plants that experienced 10 days of drought, while K, Co, and Mn efficiently increased in plants undergoing a prolonged drought period. There was a significant decrease in Nickel minerals in plants exposed to a 15-day water deficit period. Conversely, a significant increase was observed in Ni, P, K, Co, and Mn minerals in plants that have been through a 15-day drought. Drought periods showed no clear change in Cobalt uptake and accumulation. The results indicate that plants accumulate more minerals when experiencing drought.

The comparison between different cultivars and treatments indicates that the application of SA and FA can affect the mineral accumulation in the two cultivars studied. Specifically, the response of MAY 505 to treatments was completely different from that of MAY 455 under similar growing conditions. The highest increase in P and Cu was observed in plants treated with (1 mM SA + 10 mM FA), while Ni levels were highest in plants treated with (5 mM FA). In contrast to MAY 455, the cultivar MAY 505 showed a significant increase in Mn and K in plants sprayed with (2 mM SA). Each of N and Co recorded the highest accumulation with (2 mM SA + 10 mM FA). This suggests that different treatments can lead to diverse outcomes for both cultivars. When considering the interaction between cultivar and drought, it is evident that the impact of treatments is more pronounced after 15 days of drought compared to 10 days of drought and control plants. This indicates that plants adapt their physiological processes to enhance mineral accumulation during periods of drought.

Discussion

Proline Accumulation

This study attempts to unveil the role of folic and salicylic acids on the physiological attributes of cotton plants under drought conditions. The findings of this study supported that proline accumulation occurs under prolonged drought periods. It has been reported that proline production increases when plants are undergoing drought periods (D'Oria *et al.*, 2024; Masheva *et al.*, 2022; Abbas *et al.*, 2021). The exogenous application of 2 mM SA led to the alleviation of drought stress by increasing γ-glutamyl kinase (GK) which in turn increased proline production (Nazar *et al.*, 2015). Furthermore, the exogenous application of 10 mM FA significantly increased proline accumulation under drought conditions. Both cotton cultivars lessened the detrimental effects of drought and increased the accumulation of proline through a significant increase in proline content. Since proline is considered an osmolyte and helps sustain cellular turgor pressure and protect proteins from degradation (Blum, 2017; Chen & Zhang, 2016).

In this study, the cultivar-specific responses to drought showed variability among the two studied cultivars. Specifically, MAY 505 was shown to accumulate more proline than MAY 455, suggesting its superior tolerance to drought. Such variability in cultivars was observed in other studies (Rosales *et al*., 2012). On the other hand, the combined impact of SA/FA on proline showed more effect on proline accumulation. This finding corresponds to other studies that collectively suggest the pivotal role of such compounds in signaling pathways that regulate the synthesis of proline (Hayat et al., 2010; Itaf et al., 2023).

Chlorophyll content

Chlorophyll pigments which play an important role in the process of photosynthesis, can change under abiotic stress. In this study, chlorophyll content was shown to decrease with increased drought period which is consistent with previous studies (Li *et al*., 2022; Nikolaeva *et al*., 2010). In this regard, cultivar MAY 455 was less affected by drought periods when compared to MAY 505. This indicates cultivar MAY 455 adapts more efficient mechanisms for protecting the machinery of photosynthesis under water deficit conditions.

Although the application of SA/FA treatments has a significant impact on the accumulation of proline, oil content, and mineral uptake, no prominent effect was observed in chlorophyll content (Figure 10).

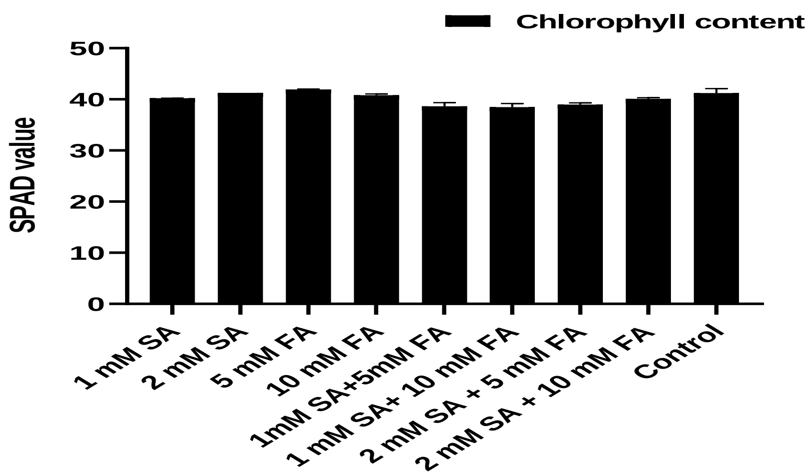

Figure 10. The impact of treatment on chlorophyll content.

The data presented in (Figure 11) showed a significant change between the two studied cultivars and among the treatments applied. Thus, the results from the interaction between cultivar vs treatment revealed that cultivar MAY 455 recorded the highest SPAD readings at (44.92) when plants were sprayed with 5 mM FA. However, less chlorophyll content was observed in MAY 505 at only (38.88) under similar conditions. On the contrary, when the plant was sprayed with 10 mM FA, MAY 505 produced more chlorophyll than MAY 455 under similar growth conditions. These findings align with those found by (Al-Hashimi and Zeboon, 2024A; Asma *et al*., 2023). These findings indicate that the cultivar response to the treatments varied. Therefore, there is no clear pattern on the effect of treatment on chlorophyll content.

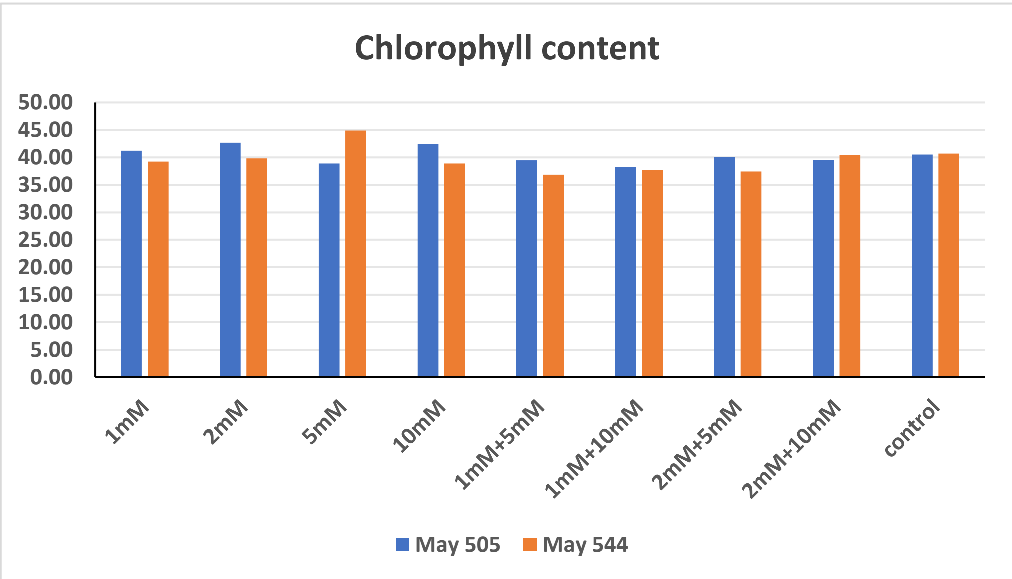

Figure 11. shows the interaction between cultivar vs treatment in cotton plants.

Oil Content

Another important aspect of physiological aspects is the production of oil. The findings of this study reveal the direct impact of drought stress on oil content in the two studied varieties. These findings aligned with observations on safflower (Joshan *et al.,* 2019). Nevertheless, during this study, it was observed that MAY 505 constantly produced more oil than MAY 455 plants, indicating its adaptations to drought stress (Li *et al.*, 2022).

Drought stress can significantly affect the production of cotton oil. The foliar spray of SA and FA under water deficit did not enhance cotton oil productivity. In this study, oil production recorded the highest levels when plants were treated with (1 mM SA + 5 mM FA) among all other treatments applied. Nonetheless, the effect of treatments on cotton oil yield was shown to have a similar effect but the combined treatments of SA/FA showed higher yield as among other treatments. These findings suggest that further investigations are required to better understand the potential effects of combined SA/FA treatment to optimize cotton oil synthesis under drought stress. However, the production of cotton oil decreased with increased drought periods due to the lack of water, insufficient carbon sources, and decreased enzyme activities (Li *et al*., 2022).

Mineral accumulation

Mineral movement and transportation are vital in plant physiology. Drought stress can significantly restrict minerals' mobility in soil and plant tissues (Li *et al*., 2023). The current study demonstrated the influence of drought on some physiological parameters. When investigating minerals, cultivar MAY 505 showed increased accumulation of minerals such as K and Co, while cultivar MAY 455 increased the buildup of Cu and Ni. These findings indicate that these minerals play a crucial role in providing tolerance against drought.

The data presented in this study demonstrated that the accumulation of Co, N, P, and Mn increased which comes in agreement with the results obtained by (Ahanger *et al*., 2016). On the other hand, a decrease was observed in the uptake and accumulation of Cu and K which coincides with data obtained by (D'Oria *et al*., 2022; Samarah *et al.,* 2004). Regardless of the drought effect, the spray of SA/FA enhanced the uptake and accumulation of minerals (Shan *et al*., 2024; Itaf *et al.*, 2023). Moreover, the foliar spray a combined SA and FA resulted in a higher accumulation of minerals than the application of these hormones individually. Thus, these compounds promoted cotton plants in better acquisition of minerals under drought stress.

Total soluble sugars

Plants under stressed environments lead to the accumulation of total soluble sugars (TSS) which act as osmoprotectants thereby cellular turgor and vital metabolic processes are sustained. This study confirmed that levels of TSS were significantly increased under drought stress. The elevated levels of TSS help cells protect their osmotic balance, structure, and antioxidant activities which collectively increase a plant's potential against drought stress (Blum, 2017).

The results of the current study revealed that both cultivars significantly increased levels of TSS under drought stress, especially after 15 days of drought. On the other hand, both cultivars upon the exogenous application of SA and FA whether individually or combined increased the buildup of TSS. Further, both cultivars showed the highest accumulation of TSS when plants were treated with 2 mM SA and a combined treatment (2 mM SA+10 mM FA) under 15 days of drought. This result implies that there is a synergistic effect between these compounds in addressing drought conditions. Positive effects of FA and SA in mitigating drought tolerance were reported in the previous studies supporting the findings of this study (Hayat *et al*., 2010; Feng *et al.,* 2023).

The interaction impact between drought stress and foliar application of treatments generally revealed evident enhancement in physiological features such as TSS buildup. This suggests that treatment combination can be applied as a promising strategy cotton plants resistance to tolerate water deficit.

Conclusion

The current study offered important insights into the physiological changes experienced by two cotton cultivars under various water deficit conditions. Physiological aspects improved upon the application of FA and FA. Foliar-applied SA and FA significantly reinforced the accumulation of proline and decreased oil contents in both cultivars studied. Further, it led to better uptake and accumulation of N, P, K, Mn, Co, and Ni minerals. In a prolonged drought period, plants accumulated the highest amount of TSS to resist the undesirable effects of drought. Furthermore, MAY 505 produced more chlorophyll pigments than MAY 455 under similar environmental conditions. In general, MAY 505 was more responsive than MAY 455 in addressing water deficit periods.

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